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The orphan nuclear receptor SXR coordinately regulates drug metabolism and efflux

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Cytochrome P450 3A4 is an important mediator of drug catabolism that can be regulated by the steroid and xenobiotic receptor (SXR). We show here that SXR also regulates drug efflux by activating expression of the gene *MDR1*, which encodes the protein P-glycoprotein (ABCB1). Paclitaxel (Taxol), a commonly used chemotherapeutic agent, activated SXR and enhanced P-glycoprotein–mediated drug clearance. In contrast, docetaxel (Taxotere), a closely related antineoplastic agent, did not activate SXR and displayed superior pharmacokinetic properties. Docetaxel's silent properties reflect its inability to displace transcriptional corepressors from SXR. We also found that ET-743, a potent antineoplastic agent, suppressed *MDR1* transcription by acting as an inhibitor of SXR. These findings demonstrate how the molecular activities of SXR can be manipulated to control drug clearance.

The effectiveness of many pharmacologic agents is limited by metabolic inactivation and excretion. Paclitaxel (Taxol)[1], one of the most commonly used antineoplastic agents, is subject to metabolic inactivation by the hepatic cytochrome P450 enzymes CYP3A4 and CYP2C8. Both enzymes hydroxylate paclitaxel thereby abolishing the drug's antimitotic properties[2]. In addition to being inactivated by hepatic P450 enzymes, paclitaxel is excreted from the intestine via P-glycoprotein (ABCB1), a broad-specificity efflux pump protein encoded by the gene *MDR1* (also known as *ABCB1*). Gene-targeting studies have demonstrated that P-glycoprotein is responsible for fecal excretion of 85% of orally administered paclitaxel. Moreover, when overexpressed in tumor cells, P-glycoprotein establishes a barrier to the uptake of paclitaxel and other agents, thereby leading to the therapeutic obstacle of multidrug resistance[3].

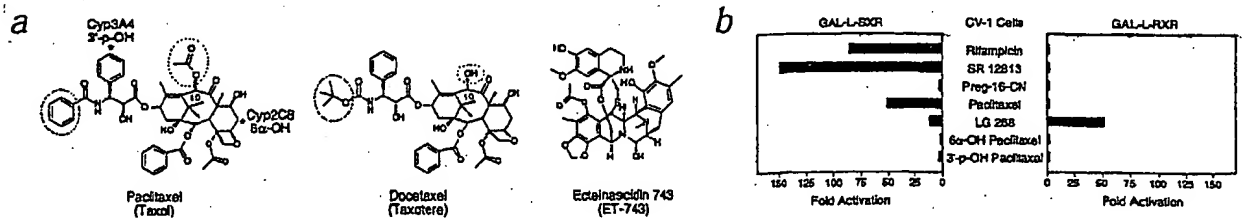
CYP3A4 is of considerable interest as this enzyme is responsible for the degradation of approximately 50% of all pharmaceutical agents and its transcription can be induced by a variety of its substrates[4]. Several recent studies demonstrate that the orphan nuclear receptor, SXR (also known as PXR, PAR, PRR or NR1I2) has a central role in regulating *CYP3A4* transcription[5,6]. SXR is activated by a diverse array of pharmaceutical agents including rifampicin, SR12813, clotrimazole, phenobarbital and hyperforin[7–10], and is thus a broad-specificity xenobiotic sensor with a central role in regulating hepatic drug metabolism. However, SXR is also highly expressed in intestine and its role there has yet to be addressed.

We show here that paclitaxel activated SXR and induced hepatic expression of *CYP2C8* as well as *CYP3A4*. We also found that SXR activated *MDR1* expression in intestinal tumor cells causing enhanced paclitaxel efflux. These results widen the repertoire of the SXR response to include additional catabolic pathways, intestinal drug excretion and multidrug resistance.

Finally, the ability of SXR to coordinately regulate multiple xenobiotic clearance pathways indicates that this receptor could be exploited to select drug candidates that either fail to activate or perhaps even inhibit these clearance pathways. We have identified drugs that exhibit both types of activities and suggest that SXR responses can be manipulated in a clinical setting.

Paclitaxel activates SXR

The ability of paclitaxel (Fig. 1*a*) to induce *CYP3A4* expression[11] prompted us to investigate whether it could activate SXR. We transfected CV-1 cells with a Gal4 reporter along with a vector expressing the ligand-binding domain of human SXR linked to the DNA-binding domain of yeast Gal4 (Gal-L-SXR). As expected, Gal-L-SXR was activated by 10 μM doses of the SXR agonists rifampicin and SR12813, but not by pregnenolone-16α-carbonitrile, a specific agonist of pregnane X receptor[12] (PXR, the mouse ortholog of SXR; Fig. 1*b*). Paclitaxel strongly activated SXR (50-fold, $EC_{50} \approx 5$ μM) at clinically relevant concentrations[13] (Fig. 1*b*). No activation was seen with the retinoid X receptor (RXR, the heterodimeric partner of SXR) ligand LG268 (100 nM) or with 3′-p-hydroxypaclitaxel or 6α-hydroxypaclitaxel, the respective products of paclitaxel metabolism by CYP3A4 and CYP2C8 (Fig. 1*b*). We obtained qualitatively similar results with wild-type SXR (data not shown). Activation of SXR by paclitaxel was specific as this compound had no effect on RXR (Fig. 1*b*), or on other nuclear receptors including PXR, estrogen receptor-α, vitamin D receptor, thyroid hormone receptor-β, retinoic acid receptor-α, FXR, LXR-α, PPAR-α, -γ, -δ or CAR-β (Fig. 1*c*), a nuclear receptor whose sequence and ligand-responsiveness are closely related to that of SXR (refs. 14,15).



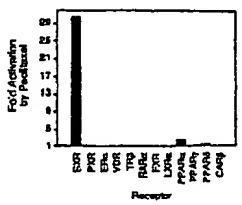


Fig. 1 Paclitaxel is a specific activator of SXR. *a*, Chemical structures of paclitaxel, docetaxel and ecteinascidin 743 are shown and the differences between paclitaxel and docetaxel are circled. The positions where paclitaxel is hydroxylated by CYP3A4 and CYP2C8 are also indicated. *b*, SXR is activated by paclitaxel. After transfection with Gal-L-SXR or Gal-L-RXR, CV-1 cells were treated with the indicated compounds and fold activation was determined relative to untreated cells. *c*, Paclitaxel specifically activates SXR. Transfections were performed as above using previously described plasmids[14,41–43]. As positive controls, each receptor was activated by its cognate ligand.

SXR induces *CYP2C8* and *MDR1* expression

Having shown that paclitaxel is an efficient and selective activator of SXR, we compared its ability to activate *CYP3A4* expression with that of other SXR agonists. We treated primary human hepatocytes with SXR agonists and monitored *CYP3A4* expression by northern-blot analysis. Consistent with the transfection experiments, rifampicin, SR12813 and paclitaxel were all effective activators of *CYP3A4* (Fig. 2*a*, left panel). We next investigated whether paclitaxel and other SXR agonists induced expression of *CYP2C8*, the other cytochrome P450 enzyme that inactivates paclitaxel *in vivo*[2,13]. In human hepatocytes, rifampicin, paclitaxel (Fig. 2*a*, left panel) and hyperforin (data not shown) strongly activated *CYP2C8* expression, whereas the RXR ligand LG268 was inactive. The fold response to SR12813 was less than that seen with other SXR agonists and varied between hepatocyte donors (Fig. 2*a*, left panel and data not shown). Nonetheless, activation by rifampicin, paclitaxel and hyperforin indicates that human *CYP2C8* is a downstream target of SXR activation.

We also investigated whether SXR could control drug efflux by regulating P-glycoprotein levels. In primary hepatocyte cultures, several SXR agonists enhanced *MDR1* expression (Fig. 2*a*, left panel). In addition to being expressed in the liver, SXR, CYP3A4 and P-glycoprotein are co-expressed in intestinal epithelial cells[3,12,16,17]. To examine SXR activity in intestinal cells, we used LS180 cells, a colon cancer cell-line that expresses SXR (data not shown). *CYP3A4* is expressed at low levels in intestinal cells but was induced by SXR ligands (Fig. 2*a*, right panel). Similarly, *MDR1* was strongly induced by the same SXR ligands (Fig. 2*a*, right panel) as well as by hyperforin (data not shown), another potent SXR ligand[8,9]. These data indicate that *MDR1* is an SXR target gene in both intestine and liver.

To further confirm the link between SXR and *MDR1*, we investigated whether a constitutively active variant of SXR could activate *MDR1* expression in the absence of SXR ligands. To construct such a receptor, we fused the constitutively active herpes virus VP16 transactivation domain to the full-length SXR (VP-SXR). Although wild-type SXR was inactive in the absence of ligand, the VP-SXR chimera constitutively activated a reporter construct containing SXR response elements from the *CYP3A4* promoter (Fig. 2*b*). When expressed in LS180 cells, unliganded VP-SXR induced expression of *CYP3A4* and *MDR1* but had little effect on the RXR-α and GAPDH control transcripts (Fig. 2*c*). The effect of VP-SXR was specific given that VP-FXR, a chimera with another nu-

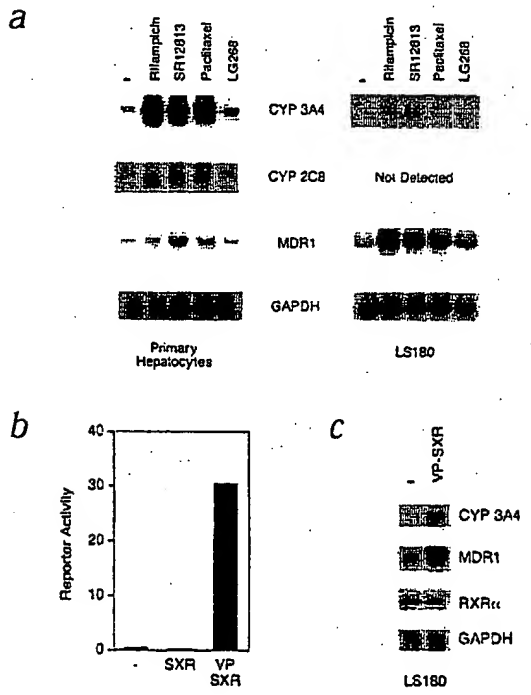


Fig. 2 SXR activates expression of *CYP2C8* and *MDR1*. *a*, Northern-blot analysis showing that SXR agonists induce expression of *CYP2C8* and *MDR1* mRNA in hepatocytes (left panel) and LS180 human colon cancer cells (right panel) treated with the indicated compounds. *b*, VP-SXR is a constitutively active version of SXR as shown by co-transfection of CV-1 cells with the SXR reporter (CYP3A4x3-TK-luc) and expression vectors for human SXR or VP-SXR. After transfection, cells were maintained in media without an SXR agonist. *c*, Northern-blot analysis showing that unliganded VP-SXR activates expression of endogenous *CYP3A4* and *MDR1* genes. LS180 cells were transiently transfected with GFP-expression vector alone (-) or with GFP and VP-SXR and maintained in media lacking SXR agonists.

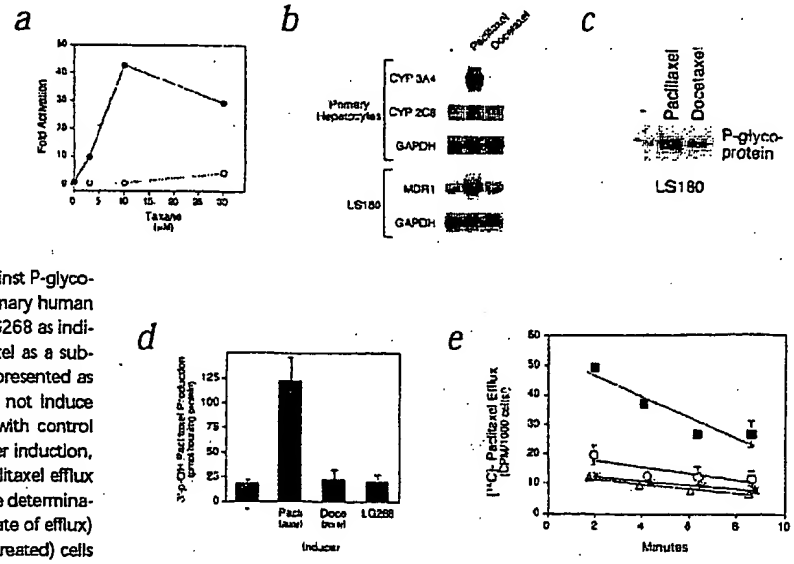


Fig. 3 Docetaxel does not activate SXR or induce drug clearance. *a*, Docetaxel does not activate SXR. Fold activation of the Gal-L-SXR reporter in CV-1 cells transiently transfected with Gal-L-SXR and treated with the indicated concentrations of paclitaxel (■) or docetaxel (○). *b*, Docetaxel does not induce expression of endogenous SXR target genes as shown by northern-blot analysis. Hepatocytes (upper panel) and LS180 cells (lower panel) were treated with the indicated compounds. *c*, Paclitaxel induces P-glycoprotein expression. LS180 cells were treated with the indicated compounds and subjected to western-blot analysis using antibody against P-glycoprotein. *d*, Docetaxel fails to induce drug metabolism. Primary human hepatocytes were induced with paclitaxel, docetaxel or LG268 as indicated, then CYP3A4 activity was measured using paclitaxel as a substrate for the production of 3'-p-hydroxypaclitaxel. Data presented as mean ± s.d. of triplicate data points. *e*, Docetaxel does not induce MDR1-mediated drug efflux. LS180 cells were induced with control media (△), paclitaxel (■), docetaxel (○) or LG268 (×). After induction, cells were loaded with [^{14}C]-paclitaxel and the rate of paclitaxel efflux was determined. Data presented as means ± s.d. of triplicate determinations; lines are lines of regression. The slope of each line (rate of efflux) was compared with the slope obtained in the control (untreated) cells using an analysis of covariance.

clear receptor, was inactive as was a VP-SXR construct that lacked the SXR DNA-binding domain (data not shown). Together, these data demonstrate that SXR regulates *MDR1* expression in the intestine.

Dissociation of SXR activation from antineoplastic activity

The ability of paclitaxel to activate SXR shows that the effectiveness of this drug might be limited by autoinduced metabolism, *MDR1*-mediated clearance and/or multidrug resistance. This implies that taxane analogs that lack SXR agonist activity might have improved pharmacokinetic properties. We thus examined the transcriptional effects of docetaxel (Taxotere), a clinically tested paclitaxel analog with similar antineoplastic activity. Docetaxel differs from paclitaxel in that it has a hydroxyl group in place of the acetyl moiety at position 10 and an N-*tert*-butoxycarbonyl group instead of the N-benzoyl group on the terminal side chain. These differences have little effect on antineoplastic potency as both taxanes inhibit microtubule depolymerization at similar concentrations[1,18]. However, these differences were critical in determining SXR responsiveness as docetaxel did not effectively activate Gal-L-SXR (Fig. 3*a*). The chemical differences between paclitaxel and docetaxel allow the cytotoxic effects of the taxanes to be dissociated from their SXR-mediated transcriptional effects. This result predicts that docetaxel cannot activate endogenous SXR-target genes. Docetaxel did not activate *CYP3A4* and *CYP2C8* mRNA expression in primary hepatocytes and did not induce *MDR1* expression in LS180 intestinal cells (Fig. 3*b*). Similarly, western-blot analysis indicated that paclitaxel was much more effective than docetaxel in inducing *MDR1* (P-glycoprotein) expression in LS180 cells (Fig. 3*c*).

To test the ability of docetaxel to regulate drug clearance, we compared the ability of the two taxane analogs to induce paclitaxel metabolism and efflux. We maintained primary human hepatocytes in control media or media supplemented with 10 μM paclitaxel or docetaxel for 48 hours. After the induction period, we removed the antineoplastic agents and measured CYP3A4 activity using paclitaxel as a substrate. Whereas paclitaxel pretreatment induced an approximately five-fold increase in the rate of 3'-p-hydroxypaclitaxel production, both docetaxel and the control RXR ligand (LG268) had no effect on CYP3A4 activity (Fig. 3*d*). To examine taxane-induced drug efflux, we pretreated LS180 colon cancer cells as above and measured the rate of drug efflux using [^{14}C]paclitaxel as a substrate for P-glycoprotein (Fig. 3*e*). The rate of drug efflux from paclitaxel-pretreated cells (3.55 cpm/1000 cells/min) was significantly faster than that from untreated cells (0.85 cpm/1000 cells/min, $P = 0.002$), whereas the rate of efflux from docetaxel (1.09 cpm/1000 cells/min, $P = 0.366$) and LG268 (0.65 cpm/1000 cells/min, $P = 0.094$) pretreated cells did not differ from controls. These data show that SXR can be used to identify drug analogs that do not induce hepatic metabolism or P-glycoprotein-mediated drug transport.

Docetaxel does not displace nuclear-receptor corepressors

Given the potential usefulness of generating SXR-transparent drugs, we investigated the molecular mechanism underlying the restricted activity of docetaxel. Ligands for nuclear hormone receptors activate transcription by initiating an exchange among coregulatory proteins that associate with the receptor[19]. In the absence of ligand, some receptors associate with a repressor complex that uses the corepressors silencing mediator of retinoid and thyroid hormone (SMRT) or nuclear receptor corepressor (NCoR) to dock to the receptor surface. Ligand binding results in a reorientation of the receptor's transactivation domain which displaces the corepressor and simultaneously recruits a number of co-activator proteins, including members of the p160 family (SRC-1, ACTR and GRIP) and PBP (DRIP205 and TRAP220)[19]. We used a mammalian two-hybrid assay to compare the effects of paclitaxel and docetaxel on coregulator recruitment. We transiently transfected CV-1 cells with a Gal4 reporter construct, a vector expressing a VP16-SXR ligand-binding domain fusion (VP-L-SXR), and an expression vector for the Gal4 DNA-binding domain or Gal4 linked to the indicated co-activator interaction domain

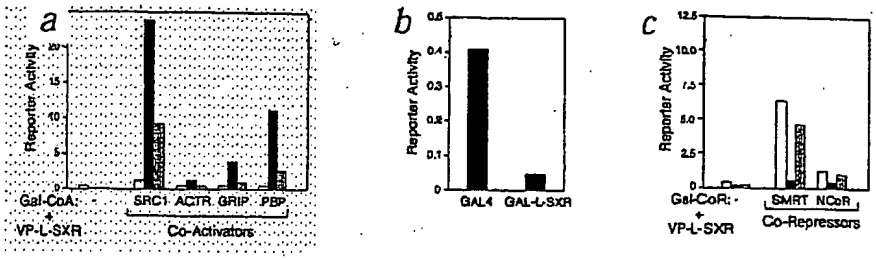


Fig. 4 Paclitaxel and docetaxel recruit co-activators but only paclitaxel displaces corepressors. *a*, Paclitaxel and docetaxel recruit co-activators to SXR. CV-1 cells were transiently transfected with a Gal4 reporter and VP-L-SXR as well as expression vectors for the Gal4 DNA-binding domain (-) or Gal4 linked to the receptor interaction domains of the indicated nuclear receptor co-activators (Gal-CoA). Cells were then treated with control media (□) or media containing paclitaxel (■) or docetaxel (▨). *b*, Unliganded SXR can repress basal transcriptional activity. CV-1 cells were transiently transfected with the Gal4 DNA-binding domain or Gal-L-SXR. Reporter activity was measured in cells maintained in the absence of ligand. *c*, Docetaxel fails to displace corepressors from SXR. CV-1 cells were transiently transfected as in *a* but the Gal-co-activator expression vectors were replaced with expression vectors for Gal4 linked to the receptor interaction domains of the indicated nuclear receptor corepressors (Gal-CoR). After transfection cells were treated with control media (□) or media containing paclitaxel (■) or docetaxel (▨).

(Gal-CoA). In this system, reporter expression is activated if VP16 becomes tethered to the promoter through an SXR–co-activator interaction[20]. Paclitaxel promoted a specific interaction with all of the co-activators tested except CBP (Fig. 4*a* and data not shown). Docetaxel promoted a qualitatively similar response though its effect was 25–40% less than that seen with paclitaxel. These findings indicate that docetaxel has the potential to act as a partial SXR agonist. However, this partial response cannot fully account for docetaxel's crippled activity on SXR.

We next investigated whether the diminished response to docetaxel reflected altered corepressor displacement. Nuclear-receptor corepressors were first identified based on their ability to interact with receptors that suppress transcription in the unliganded state (for example, thyroid hormone and retinoic acid receptors)[19]. However, subsequent studies have demonstrated that corepressors also interact with receptors that do not repress basal transcription. For example, the mixed estrogen-receptor agonist/antagonist 4-hydroxytamoxifen recruits corepressors to the estrogen receptor. In this context, corepressor recruitment does not repress basal transcription, it prevents transactivation[21–24]. We investigated whether corepressors have a role in SXR action by testing if SXR represses basal transcription. Unliganded Gal-L-SXR repressed basal transcription by approximately four-fold (Fig. 4*b*), and this result prompted us to use the mammalian two-hybrid assay to evaluate potential SXR-corepressor interactions. Unliganded SXR interacted with the nuclear corepressor SMRT and, more importantly, paclitaxel reversed this interaction whereas docetaxel had little effect (Fig. 4*c*). The SXR–NCoR interaction was significantly weaker, although the differential response of the two drugs was maintained. These data indicate that the restricted activity of docetaxel on SXR is closely related to its inability to displace corepressors.

Ecteinascidin-743 antagonizes SXR action

An alternative approach to limiting SXR-mediated clearance and/or multidrug resistance would be to develop an SXR antagonist. Recent studies have identified ecteinascidin-743 (ET-743), a novel, marine-derived natural product, as an extremely potent antineoplastic agent. This compound inhibits the proliferation of a variety of cancer cell lines and human xenografts with half-maximal inhibition (IC_{50}) measurements ranging from 1 to 100 nM (refs. 25,26). Although the mechanism of action of this drug is unclear, ET-743 has been shown to inhibit trichostatin-induced transcription of *MDR1* (refs. 27,28). The ability of a low molecular weight, hydrophobic compound such as ET-743 to regulate *MDR1* transcription

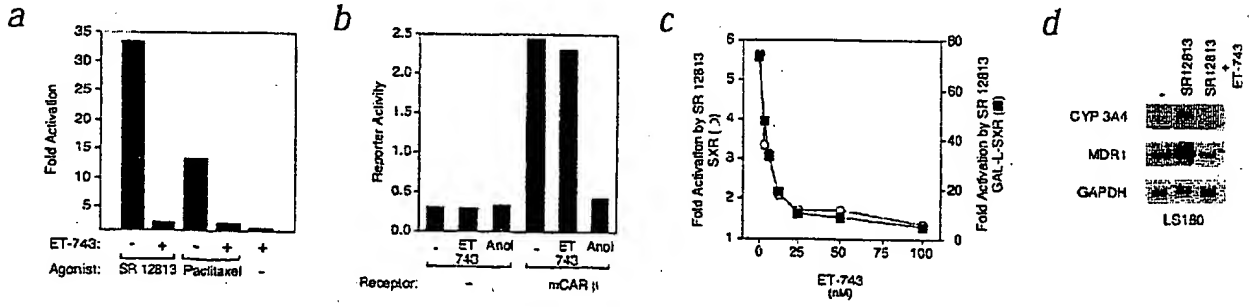


Fig. 5 Ecteinascidin 743 (ET-743) inhibits transcriptional activation by SXR. *a*, ET-743 inhibits ligand-induced activation of SXR. CV-1 cells were transiently transfected with Gal-L-SXR and treated with SR12813, paclitaxel and/or ET-743, as indicated (+ or -). *b*, ET-743 does not inhibit transcriptional activation by CAR-β. CV-1 cells were transfected with an LXREx3-TK-luc reporter and an expression vector for CAR-β, where indicated. After transfection, cells were treated with control media (-) or media containing androstanol or ET-743 as indicated. *c*, Dose response for ET-743 inhibition of SXR. CV-1 cells were transiently transfected with SXR and a CYP3A4x3 TK-luc reporter (□) or with Gal-L-SXR and a Gal4 reporter (■). Cells were then treated with control media or media supplemented with SR12813 and the indicated concentrations of ET-743. Fold activation of the reporter was determined relative to untreated cells. *d*, Northern-blot analysis showing ET-743 inhibits agonist-induced activation of SXR target genes. LS180 cells (right panel) were treated with control media or media supplemented with SR12813 ± ET-743.

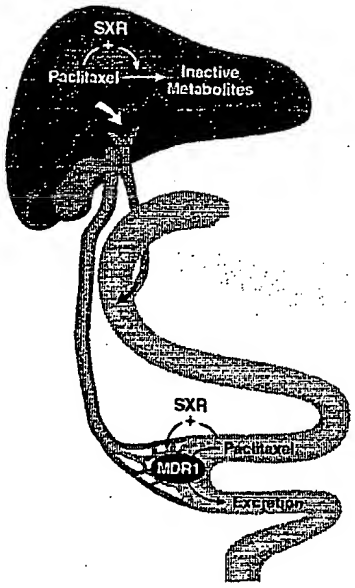


Fig. 6 SXR coordinately regulates drug metabolism and efflux. The response to a xenobiotic challenge is illustrated with paclitaxel. Oral exposure to paclitaxel results in activation of SXR in intestinal epithelial cells. This results in enhanced expression of the P-glycoprotein transporter and subsequent excretion of paclitaxel into intestinal fluid. In principle, any paclitaxel that passes this barrier could be transported to the liver through the portal vessels and eventually enter the general circulation. However, paclitaxel is hydroxylated by CYP3A4, a modification that destroys the cytotoxic properties of this drug. CYP3A4 is expressed in the intestine and liver and is induced by SXR. In addition, CYP2C8, another paclitaxel-inactivating enzyme, is also induced by SXR in the liver. The inactivated paclitaxel metabolites can be secreted into the biliary fluid and then removed from the gastrointestinal tract. Thus, in response to a xenobiotic challenge, SXR can induce both a first line of defense (intestinal excretion) and a back-up system (hepatic inactivation) that limits exposure to potentially toxic compounds. Although this system can limit exposure to environmental toxins, it can create a therapeutic problem when it limits the bioavailability of pharmaceutical compounds. Similarly, this regulatory loop could prevent killing of cells by chemotherapeutic agents should it be activated in a tumor.

prompted us to examine whether this compound could antagonize SXR. Indeed, we found that ET-743 (50 nM) was a potent inhibitor of SR12813- and paclitaxel-induced activation of Gal-L-SXR (Fig. 5*a*). In contrast, ET-743 had no effect on the transcriptional activity of CAR-β (Fig. 5*b*), a constitutively active nuclear receptor whose transcription is suppressed by androstanol and whose ligand responsiveness overlaps that of SXR (refs. 14,15).

Dose-response studies demonstrated that ET-743 maximally inhibited both wild-type SXR and Gal-L-SXR at concentrations of 25–50 nM; IC_{50} occurred at approximately 3 nM (Fig. 5*c*). This dose-response profile matches that reported for inhibition of trichostatin-induced *MDR1* transcription and for the antineoplastic effects of ET-743 (refs. 25–28). Finally, as ET-743 antagonizes SXR, it should repress ligand-mediated activation of SXR target genes. Northern-blot analysis indicated that ET-743 (40 nM) inhibited SR12813-induced activation of both *CYP3A4* and *MDR1* but had no effect on the GAPDH control (Fig. 5*d*). These data indicate that ET-743 can repress *MDR1* transcription by antagonizing SXR.

Discussion

Previous studies have demonstrated that SXR is a xenobiotic sensor that activates *CYP3A4* transcription in the liver. These findings defined a limited role for SXR in the metabolic inactivation of a subset of xenobiotic compounds. We show here that SXR agonists also activate *CYP2C8* expression in human hepatocytes. This is consistent with recent reports that rifampicin activates human *CYP2C8* expression[29] and that compounds now known to activate rodent SXR induce expression of hamster *Cyp2c28* (ref. 30). These findings indicate that SXR plays an unexpectedly broad role in regulating drug metabolism as CYP2C8 has been implicated in the degradation of a variety of clinically significant drugs including paclitaxel, azidothymidine, verapamil, ibuprofen, thiazolidinediones, benzodiazepines and others[31,32].

Although xenobiotic compounds are routinely cleared by metabolic inactivation, other mechanisms exist to purge the body of potentially toxic foreign compounds. In fact, inhibition of xenobiotic uptake would serve as a more logical first line of defense. P-glycoprotein is a broad-specificity xenobiotic transporter that inhibits uptake and subsequent exposure to a wide variety of foreign compounds[3]. Using a combination of pharmacologic and genetic approaches, we showed that SXR activated *MDR1* expression in hepatocytes and intestinal cells and that this activation resulted in enhanced drug efflux. This is the first evidence that SXR coordinately regulates multiple xenobiotic clearance pathways (metabolism and excretion) in different tissues (intestine and liver). Interestingly, SXR and P-glycoprotein are co-expressed in a number of other tissues including the kidney[3,7,17] and placenta[33]. This indicates that SXR might regulate renal drug excretion as well as fetal exposure to xenobiotics. Thus the development of specific SXR agonists might provide a pharmaceutical approach to limit developmental disorders resulting from excessive xenobiotic exposure. P-glycoprotein is also expressed in capillary endothelial cells of the blood–brain and blood–testis barriers[3]. It will be interesting to determine whether SXR is also expressed in these protected spaces.

Paclitaxel has a broad spectrum of antineoplastic activity and is perhaps the most important chemotherapeutic agent to be developed in the past two decades. Previous studies have demonstrated that *in vivo*, paclitaxel is metabolized by CYP3A4 and CYP2C8 and is excreted by P-glycoprotein. We show here that paclitaxel activates SXR at concentrations ($EC_{50} \approx 5\ \mu M$) that are clinically relevant[11,13,34] and which match the *Km* for degradation of paclitaxel by CYP3A4 and CYP2C8 ($K_m \approx 10\ \mu M$)[2,13]. Activation of SXR by paclitaxel results in enhanced expression of CYP3A4, CYP2C8 and P-glycoprotein. This regulatory loop is significant as P-glycoprotein is highly effective in preventing paclitaxel uptake from the intestine[17] (Fig. 6). Any paclitaxel that does enter the bloodstream is ultimately subject to hepatic metabolism (CYP3A4 and CYP2C8) and biliary excretion (P-glycoprotein), both of which are induced by SXR. Interestingly, paclitaxel-induced clearance is self-limited as the products of paclitaxel metabolism by CYP3A4 and CYP2C8 do not activate SXR (Fig. 1*b*).

Overexpression of *MDR1* is highly problematic in cancer chemotherapy as it leads to the development of drug-resistant tumors[3]. Because it can induce *MDR1* expression, SXR could potentially promote resistance to any chemotherapeutic agent that is a substrate for P-glycoprotein. For example, we showed here that paclitaxel induces its own efflux from LS180 colon cancer cells (Fig. 3*e*). Thus, in addition to regulating traditional drug clearance pathways, SXR might also regulate multidrug resistance in SXR-expressing tumors. A comprehensive analysis of

SXR expression in various tumors has not been conducted, although SXR has been detected in neoplastic breast tissue[35] and in colon-cancer-derived cells (Fig. 3 and data not shown). Future classification of tumors as 'SXR-positive' or 'SXR-negative' might help predict whether the tumor is likely to develop chemotherapy-induced drug resistance.

The ability of a drug to induce SXR-mediated clearance could limit the therapeutic potential of the inducing drug as well as that of co-administered compounds. In fact, enhanced paclitaxel clearance has been reported in patients treated with phenobarbital[36–38], a compound known to activate SXR (ref. 10). This type of drug–drug interaction can be particularly problematic in cancer chemotherapy where combination therapies are widely used. This implies that 'SXR-transparent' drugs might offer therapeutic advantages to their SXR-inducible counterparts. We found that the taxane analog docetaxel did not activate SXR or SXR-mediated drug clearance (Fig. 3). The SXR-transparent properties of this drug could not be accounted for by an inability to recruit co-activator; rather, the drug failed to displace corepressors (Fig. 4). Thus, the chemical differences between paclitaxel and docetaxel (Fig. 1*a*) define a pharmacophore that can be selectively manipulated to minimize SXR responsiveness. Based on our findings, we predict that docetaxel, an SXR-transparent drug, should have improved pharmacokinetic properties. In fact, clinical studies have shown that docetaxel has a longer plasma and intracellular half-life than paclitaxel[1,18].

MDR1 expression can be a significant barrier to effective treatment in cancer chemotherapy. In addition to its effects on drug efflux, recent studies indicate that P-glycoprotein might directly inhibit cells from undergoing apoptosis[39,40]. Thus, in addition to developing SXR-transparent drugs, there could be significant therapeutic value in identifying SXR antagonists that inhibit *MDR1* expression. We found that ET-743 antagonizes SXR at nanomolar concentrations. Previous studies have shown that nanomolar doses of ET-743 inhibit transcriptional activation of *MDR1* (refs. 27,28). It has been suggested that this reflects an inhibition of DNA-binding activity by the transcription factor nuclear transcription factor-Y (NF-Y). However, it is not clear if this is a direct effect of ET-743; inhibition of NF-Y binding required approximately 1000-fold higher levels of ET-743. Our findings provide the first evidence for a molecular target for ET-743 that responds appropriately at nanomolar concentrations. The identification of a compound that inhibits SXR-mediated drug-clearance pathways points to a molecular approach of developing pharmaceutical reagents that enhance therapeutic efficacy. This might eventually allow for the use of lower doses of conventional chemotherapy so as to minimize the cytotoxic side effects of these drugs.

We show that SXR coordinately regulates a network of xenobiotic clearance genes in the liver and intestine and that SXR can be used to identify compounds that differentially modulate these drug clearance pathways. SXR is thus the Achilles' heel of drug clearance and an ideal molecular target for the manipulation of this signaling network.

Methods

Plasmids and reagents. A cytomegalovirus expression vector was used to express the following proteins: human SXR (accession #AF061056); Gal-L-SXR (human SXR ligand-binding domain, Lys 107 to Ser 443); Gal-SRC1 (human SRC-1, #U59302, Asp 617 to Asp 769); Gal-ACTR (human ACTR, #AF036892, Ala 616 to Gln 768); Gal-GRIP (mouse GRIP1, #U39060, Arg 625 to Lys 765); Gal-PBP (human PBP, #AF283812, Val 574 to Ser 649); Gal-SMRT (human SMRT, #U37146, Arg 1109 to Gly 1330); Gal-NCoR (mouse NCoR, #U35312, Arg 2065 to Gly 2287); VP-SXR (full-length human SXR); and VP-L-SXR (human SXR ligand-binding domain, Lys 107 to Ser 443). Gal4 fusions containing the indicated fragments were fused to the C-terminal end of the yeast Gal4 DNA-binding domain; VP16 fusions contained the 78 amino acid herpes virus VP16 transactivation domain. All other constructs were constructed as described[20]. Docetaxel was from Rhone-Poulenc Rorer (Collegeville, Pennsylvania); 3′-p-hydroxypaclitaxel and 6α-hydroxypaclitaxel were from Gentest (Woburn, Massachussets); rifampicin, pregnenolone-16α-carbonitrile and paclitaxel were from Sigma; and ET-743 was from the NCI Drug Synthesis and Chemistry Branch.

Transient transfection assay. CV-1 cells were grown and transiently transfected by lipofection as described[20]. LS180 cells were transfected with lipofectamine (Life Technologies, Rockville, Maryland). Unless indicated otherwise, ligands were used at the following concentrations: 10 μM rifampicin, SR12813, pregnenolone-16α-carbonitrile (Preg-16-CN), paclitaxel, docetaxel, 6α-hydroxypaclitaxel and 3′p-hydroxypaclitaxel; 5 μM andsorstanol; 100 nM LG268; and 40–50 nM ET-743. The cytotoxic effects of paclitaxel, docetaxel and ET-743 were minimal when exposure was limited to 16–24 h.

Northern-blot analysis. Primary human hepatocytes were obtained from Clonetics (Walkersville, Maryland) and cultured according to the vendor's instructions. Cells were treated with the indicated SXR agonists for 48 h and total RNA was isolated using the Trizol reagent. Human LS180 cells were maintained in Eagle's MEM supplemented with 10% FBS, 1 mM sodium pyruvate, 2 mM L-glutamine, non-essential amino acids, 50 U/ml penicillin G and 50 μg/ml streptomycin sulfate. 1 day prior to treatment LS180 cells were switched to phenol-red-free media containing 10% resin-charcoal stripped FBS and then treated for 24 h with the indicated compounds. Where indicated, GFP-positive LS180 cells were isolated using a MoFlo flow cytometer (Cytomation, Fort Collins, Colorado). Northern blots were prepared from total RNA and analyzed with the following probes: *MDR1* (accession #NM_000927, nucleotides (nt) 843–1111); *CYP2C8* (#NM_000770, nt 700–888); *CYP3A4* (#M18907, nt 1521–2058); *RXR-α* (#X52773, nt 738–1802); and *GAPDH* (#NM_002046, nt 101–331). Note that the *CYP2C8* probe was specific as it did not cross-hybridize to the two most closely related members of the *CYP2C* family: *CYP2C9* and *CYP2C19* (data not shown).

Western-blot analysis. Membrane fractions (20 μg protein/lane) from LS180 cells were separated by SDS–PAGE, transferred to PVDF membranes and probed with a 1:500 dilution of a P-glycoprotein antibody (Ab-1, Oncogene Research Products, Boston, Massachusetts) followed by a 1:1000 dilution of horseradish peroxidase-conjugated secondary antibodies against rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, California). Immunoblots were developed using a chemiluminescent detection system (Amersham).

Paclitaxel hydroxylase assays. Primary human hepatocytes were treated with the indicated drugs for 48 h to allow for accumulation of SXR-induced proteins. Cells were then washed and incubated for an additional 1 h in fresh media to allow for efflux of intracellular drug. This step effectively removed the inducer as the levels of paclitaxel and its metabolites measured in the media following this 1-hour wash step was less than 6% of the final amounts determined from CYP3A4 activity assay. Fresh media containing 10 μM paclitaxel was added for an additional 3 h and media was then collected and assayed for 3′-p-hydroxypaclitaxel by HPLC. Results were normalized to pmol of 3′-p-hydroxypaclitaxel formed per hour per mg protein.

Paclitaxel efflux assays. Following a 48-hour induction with the indicated drugs, LS180 cells were washed and incubated for an additional 1 h in fresh media to allow for efflux of intracellular drug. The cells were then incubated in media containing 10 μM [^{14}C]paclitaxel (4.9 μCi/μmol; Moravek Biochemicals, Brea, California) for 15 min and then rapidly centrifuged through silicone oil to remove all traces of extracellular radioactivity. Cells were then resuspended in fresh media and cell counts determined. At multiple time points over the next 10 min, triplicate aliquots of the cell suspension (~1 × 10^6 cells/aliquot) were again centrifuged through silicone oil and the radioactivity in the cell pellet was measured by quench-corrected liquid

scintillation counting. The rate of [^{14}C]paclitaxel efflux was determined as the slope of the [^{14}C]paclitaxel versus time plots using all data. The slope for each inducer was compared to the slope obtained in the control (untreated) cells using an analysis of covariance.

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